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Gut microbiome associated dysbiosis: Limited regimens and expanding horizons of phage therapy

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ABSTRACT

Human gut microbiota plays an important role in health, broadly influencing metabolism to the immune system and drug resistance to pathogenic colonization. Since antibiotic resistance is on the rise, and wide-spectrum antibiotics are known to have deleterious effects on microbial biodiversity targeted therapeutic interventions must be made. Bacteriophages are viruses that are commonly recognized to have a high level of specificity, targeting only the intended bacterial species without disrupting the overall microbial community. Advancements in genomics, bioinformatics, and synthetic biology led us to the identification and design of phages, capable of precisely targeting specific pathogens. In this review article, we aim to discuss both the challenges and opportunities of integrating phage therapies into clinical practice, discussing the limitations of traditional therapy as it pertains to the manipulation of the gut microbiome.

1. Introduction

A microbiota is composed of microorganisms that live in an environment, whereas a microbiome consists of the microbes themselves as well as their genetic material and their interactions with the surrounding environment such as gut. "*All disease begins in the gut*", a quote attributed to the ancient Greek physician Hippocrates nearly 2500 years ago, suggesting the correctness of the ancient knowledge. Our body and gut are inhabited by trillions of microbes, and, surprisingly, bacterial cells outnumber human cells by a factor of ten (Zmora et al., 2018; Kho and Lal, 2018). A metagenomics study reveals that the human gut microbiome contains 3.3 million genes, 150 times more than our own genome (Arumugam et al., 2011). There are almost 1000 bacterial species in our gut and most of them are predominantly *Firmicutes* and *Bacteroidetes* (Gill et al., 2006) (Human, 2012; Lozupone et al., 2012; Turnbaugh et al., 2009). This bacterial community also plays a central role in maintaining normal physiology by enriching metabolic functions, protecting against pathogens, and enhancing the immune system. Gut microbiome dysbiosis is the alteration of the structural and functional composition of the microbiome residing in the gut and is associated with an array of disorders and diseases, such as irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), colorectal cancer (CRC), *Clostridium difficile* infection (CDI), obesity, and many other neurological disorder (Afzaal et al., 2022) (Fig. 1). Since gut microbiome dysbiosis has been identified as an association between disease and, strategies to modulate gut microbiome function have been intensively studied (Hadrich, 2018) e.g. via administration of probiotics (Goldin et al., 1996; Everard et al., 2013), prebiotics (Everard et al., 2011; Grimaldi et al., 2018), diet interventions (So et al., 2018; Hughes and Holscher, 2021) and fecal microbiota transplantation (FMT) (Groen and Nieuwdorp, 2017; Chehri et al., 2018).

On the other side, "gut-virome", is the least discussed topic, playing a parallel important role in maintaining a healthy microbiome (Minot et al., 2011). As therapeutic tools, phages have been used since their discovery a century ago, despite their success in the first trials conducted by Felix d'Herèlle in 1921 in patients with dysentery, phage therapy was highly controversial and was not widely accepted (Moelling et al., 2018). Further, after the 1930s, the era of antibiotics emerged leading to

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the complete neglect of phage therapy. Nevertheless, the Eliava Institute of Bacteriophages, Microbiology, and Virology (Georgia), established in 1923, is considered one of the leading organizations in the study and production of phages as well as their practical application. However, these treatments are not regulated in Western countries, even though they are currently being used as compassionate treatment. Bacteriophages are preferable over antibiotics due to their broad-spectrum activity and replicable nature. Since systemic concentrations of antibiotics continue to decrease over time, bacteria can still multiply in the body. Thus, it is necessary to calibrate the effective workable dose of phages for clinical applications (Lin et al., 2017).

Phage survives in two types of life cycles: lytic and lysogenic. In contrast, virulent phages follow a purely lytic life cycle, whereas temperate phages follow both the lytic and the lysogenic pathway (Abedon, 2011). In the lytic life cycle, phage invades the host and exploits the bacterial cell machinery to reproduce new phage particles within 30–60 min. These phage particles lyse to infect other cells thereafter and release many more phages into the system. Unlike the lytic life cycle, the lysogenic life cycle involves the integration of the phage genome into the microbial host genome as a prophage or as a stable extra-chromosomal genetic element (Wang, 2006; Shao and Wang, 2008; St-Pierre and Endy, 2008). When an appropriate environment is present, a prophage can be induced, thereby initiating the lytic life cycle, and releasing phage particles (St-Pierre and Endy, 2008). Prophage induction can be triggered by environmental changes causing cellular stress such as antibiotics, certain nutrients, and variations in pH and temperature (Goerke et al., 2006; Allen et al., 2011; Hu et al., 2021). Due to known interaction with bacteria, these phages can influence the overall composition of the gut microbiome (Henrot and Petit, 2022). Recent studies have shown that fructose-enriched diets and SCFAs can induce prophages in lactobacilli (Oh et al., 2019).

Lytic phages are the top choice of researchers as temperate phages are more prone to acquire pathogenic traits or antibiotic resistance determinants through horizontal gene transfer. Nevertheless, advances in genetic engineering suggest that genetically modified temperate phages can be used therapeutically to inhibit the transcription of bacterial virulence factors. Several studies have indicated that temperate phages dominate the gut phage community (Breitbart et al., 2003; Reyes et al., 2010), following the "piggyback-the-winner" model for the interaction dynamics between bacteria and phages (Knowles et al., 2016; Moreno-Gallego et al., 2019). However, it is imperative to conduct a comprehensive review. Therefore, in this article, we systemically summarize the limitations of routine therapeutic approaches in manipulating gut microbiome and recommend different types of phage therapies to manipulate gut microbiome and ongoing and/or past successful clinical trials. We have also included current trends and research and associated challenges of phage therapy. We believe that this information will be useful to both basic research scientists and clinicians to gain a better understanding of how bacteriophage can be used to manipulate the gut microbiome precisely and develop alternative therapies.

2. Dysbiosis microbiome associated with diseases

The prevalence of chronic diseases such as inflammatory bowel disease (IBD), obesity, diabetes, and cardiovascular diseases is increasing sharply (Ng et al., 2014, 2017; Schnabel et al., 2015; Menke et al., 2015). The genetic and environmental factors mainly drive these chronic diseases, but recent studies indicate dysbiosis, an imbalance in microbial composition and functions, can enhance the risk (Hand et al., 2016; Blaser and Falkow, 2009; Lynch and Pedersen, 2016; Hawrelak and Myers, 2004). Typically, dysbiosis can impact the onset of chronic diseases in three ways. First, gain of function dysbiosis which is distinguished by alteration in microbiome composition resulting in the

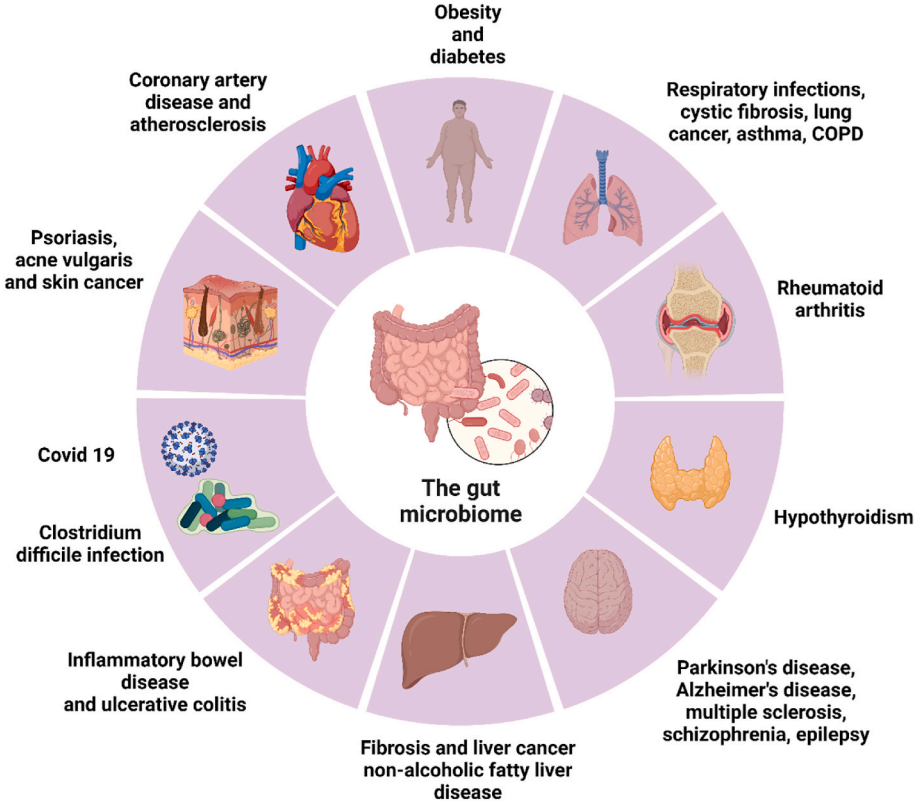

Fig. 1. Impact of gut microbial dysbiosis in human diseases. Gut microbiomes play an important role in the proper functioning of many organs, including the liver, kidneys, lungs, brain, and heart. The disruption of microbiota homeostasis leads to the malfunction of these organs, and the progression of numerous diseases (Created with BioRender.com).

acquisition of microbial functions responsible for the disease development. Gain of function dysbiosis is generally associated with infectious diseases including cholera and streptococcal pharyngitis but can also cause chronic inflammation (Liu et al., 2018; Karin et al., 2006; Medzhitov, 2008). Second, loss of function dysbiosis where the health-protective bacteria and their functions are impaired leads to the onset of chronic diseases such as IBD, obesity, and urinary stone disease (USD) (Turnbaugh et al., 2009; Sokol et al., 2009; Suryavanshi et al., 2016). Third and last, a combination of gain and loss of function dysbiosis may be implicated in the onset of disease such as recurrent infection with *Clostridum difficile* (Britton and Young, 2014). Despite the distinct association of diverse diseases with dysbiosis, in several diseases, it remains to be determined whether dysbiosis triggers disease development or is a consequence of modulations in the patient's immune system, diet, and metabolism. The contribution of dysbiosis to disease development can be determined in several ways including human prospective cohort studies, interventional trials, and mouse preclinical studies with microbiome transplantation into germ-free mice. Since microbiota has a significant impact on the host's immunity, dysbiosis may contribute to different diseases as discussed here.

2.1. Inflammatory bowel disease (IBD)

IBD including Crohn's disease (CD) and ulcerative colitis (UC) are a group of chronic gastrointestinal inflammatory conditions affecting millions of individuals in the world (Kaplan, 2015). Multiple factors impact the development of IBD including dysregulated immune responses, genetic mutations, and environmental factors (Leone et al., 2013). Recent studies have demonstrated that perturbation in the intestinal microbiome plays a central role in IBD pathogenesis, and these patients have reduced microbial diversity with an elevated ratio of Proteobacteria to *Firmicutes* in comparison to healthy subjects (Frank et al., 2007; Gevers et al., 2014; Kostic et al., 2014). However, it is still debatable whether microbial dysbiosis is one of the driving factors for inflammation in IBD patients or is just the consequence of disruption in intestinal homeostasis (Ni et al., 2017). The differences in the microbial composition between IBD patients pose a tremendous challenge in deciphering the implication of specific bacterial species in IBD pathology. Furthermore, examining the changes in functional aspects of the microbiome could be more relevant for IBD pathology in comparison to compositional modulations. Conforming to this, dysbiosis in IBD is associated with a reduction in butyrate-producing bacteria resulting in decreased butyrate levels and impaired epithelial barrier integrity leading enhanced bacterial infiltration (Machiels et al., 2014; Levy et al., 2017). Resolving the complex microbiome into well defined "symbiotic" or "dysbiotic" categories can potentially help in diagnosing modulation of host-microbiota homeostasis and advance the fecal microbiota transplant (FMT) efforts for the treatment of IBD patients.

2.2. Irritable bowel syndrome (IBS)

IBS is a functional gastrointestinal complication in industrialized countries which is diagnosed with recurrent abdominal pain related to defecation and alteration in frequency and appearance of stool (Ford et al., 2018; Lacy et al., 2016). The prevalence of IBS is estimated to be 5 to 20% in the general population (Drossman, 2006) and its pathogenesis is influenced by several factors including genetic factors (Makker et al., 2015), intestinal immune system activation (Powell et al., 2017), gut-brain axis (Moloney et al., 2016), stress (Pellissier and Bonaz, 2017), intestinal epithelial integrity (González-Castro et al., 2017) and gut microbiome (Sundin et al., 2017). It has been reported that the modulation of intestinal microbial composition and biodiversity significantly impacts IBS pathogenesis (Menees and Chey, 2018). Gut dysbiosis in IBS is associated with enhanced intestinal permeability (Fukui, 2016), activation of the intestinal immune system (Ö et al., 2010), chronic inflammation (Shi et al., 2017), anxiety, and depression (Moser et al., 2018). Several reports have tried to define dysbiosis in IBS pathology and identify specific microbiota alterations between IBS patients and healthy individuals (Casen et al., 2015; Jalanka et al., 2015). In IBS with diarrhea (IBS-D) patients, the beneficial genera *Faecalibacterium* and *Bifidobacterium* were reduced whereas the microbiota considered to be harmful such as *Bacteroides* genus and *Lactobacillaceae* and *Enterobacteriaceae* families were elevated (Pittayanon et al., 2019; Zhong et al., 2019; Rangel et al., 2015). Additionally, acute infectious gastroenteritis increases the risk of developing IBS (Halvorson et al., 2006). Small intestine of IBS patients displays bacterial overgrowth and prevention of bacterial overgrowth helps in resolution of IBS (Chen et al., 2018). Furthermore, modulating gut microbial composition with immunoglobulins, antibiotics, prebiotics, probiotics, and FMT helps in recovery of IBS patients (Valentin et al., 2017; Li et al., 2016; Ford et al., 2014; Zhang et al., 2016; El-et al., 2018). Using antibiotics for the treatment is not ideal since it perturbs the microbial biodiversity and restoration of which might take years (Jernberg et al., 2007). The concept of dysbiosis in IBS is not very well established as a large fraction of IBS patients display normal microbial composition (Jeffery et al., 2012, 2016). To estimate the intestinal microbial modulation in IBS, different methods have been employed leading to conflicting findings and making it difficult to summarize.

2.3. Diabetes

2.3.1. Type 1 diabetes (T1D)

T1D is a chronic autoimmune disease caused by the loss of insulin-producing β cells in the pancreas. Multiple studies have suggested that the oral and fecal microbiota composition in T1D patients appears to be distinct. The impact of microbiota on T1D development was first reported in 1987 by Suzuki et al. (1985). The modulations in the gut community such as reduced bacterial diversity reported to happen post-seroconversion of T1D patients and precede the acquisition of diabetic symptoms (Kostic et al., 2015) which indicate that the intestinal microbiota may contribute to triggering autoimmunity. Further, in T1D patients, the abundance of SCFAs producing bacteria appears to be reduced and supplementation with dietary acetate and butyrate drives beneficial immunological effects and protection from T1D (De Goffau et al., 2013; Mariño et al., 2017). Consistent with this, a longitudinal analysis of gut metagenomics showed that the expression of microbial genes regulating the generation of SCFAs was decreased in children who develop T1D in comparison to respective controls (Vatanen et al., 2018). Importantly, probiotic supplementation of infants within 27 days of birth decreased the risk of T1D (Vatanen et al., 2018). The investigations on the relationship between microbiota and T1D development are primarily conducted in animal models, therefore these aspects need to be validated in human subjects.

2.3.2. Type 2 diabetes (T2D)

T2D is a chronic condition affecting glucose metabolism. A condition in which the body either resists insulin, a hormone important for regulating blood sugar, or does not produce enough insulin, results in high levels of blood sugar. There has been evidence of dysbiosis in the gut microbiome in Type 2 Diabetes Mellitus (T2DM), because of alterations in the abundance of specific bacterial taxa. The presence of *Bacteroides caccae, Clostridium hathewayi, Clostridium ramosum, and Clostridium symbiosum* is higher in individuals with T2DM. Conversely, butyrate-producing bacteria such as *Ruminococcus* and *Roseburia* species tend to be depleted in patients diagnosed with T2DM1. In addition, several bacteria are believed to play a protective role by reducing proinflammatory markers and maintaining intestinal barrier integrity. For example, *Lactobacillus fermentum, Plantarum and Casei, Roseburia intestinalis, Akkermansia muciniphila* and *Bacteroides fragilis* have all been shown to improve glucose metabolism and insulin sensitivity and suppress proinflammatory cytokines. The commonly prescribed drug metformin, also known as the medication for diabetes treatment, has also

been shown to alter the intestinal microbiota, suggesting that metformin interacts with the gut microbiota through modulation of inflammation, glucose homeostasis, gut permeability, and short-chain fatty acid-producing bacteria (Lee et al., 2021). In addition, metformin promotes the production of butyrate and propionate in patients with diabetes-associated gut dysbiosis, improving a patient's capacity to catabolize amino acids (Mardinoglu et al., 2016). These changes in combination with increased levels of Akkermansia in the gut may be contributing factors to metformin's effects on glucose metabolism (Wu et al., 2017).

2.3.3. Gestational diabetes mellitus (GDM)

It is important to note that gestational diabetes mellitus (GDM), one of the most common endocrine diseases during pregnancy, is defined as any degree of glucose intolerance diagnosed during the perinatal period. Inflammation can cause insulin resistance due to physiological changes in pregnant women. The intestinal microbiome plays a crucial role in obesity and the development of insulin resistance and chronic inflammation, particularly in patients with type 2 diabetes mellitus (T2D). Numerous studies have demonstrated that intestinal dysbiosis contributes to metabolic changes in women with gestational diabetes. Most frequently, patients with GDM had an increased *Firmicutes* phylum, or decreased *Bacteroidetes* and *Actinobacteria* phyla in their microbiomes (Ionescu et al., 2022). GDM women have a low abundance of intestinal microbiota, which is associated with a proinflammatory status and insulin resistance. When compared to normoglycemic pregnant women, GDM patients had elevated concentrations of *Faecalibacterium* and *Anaerotruncus* and lower concentrations of *Clostridium* and *Veillonella*. *Bacteriodes* and *Isobaculum* were found to be in low concentrations in patients with GDM in the last trimester and the postpartum period (Crusell et al., 2018). In a study conducted by Crusell et al., higher concentrations of *Actinobacteria* phylum, *Collinsella, Rothia*, and *Desulfovibrio* genera were observed in GDM patients diagnosed in the third trimester. Additionally, alterations of the gut microbiota were still evident 8 months after birth. Women with GDM have abnormal gut microbiota that are like those of non-pregnant patients with Gut dysbiosis is still present postpartum and impacts the development of the newborn, as shown in various studies (Crusell et al., 2018).

2.4. Obesity

Obesity is an alarming health concern with a rapid increase in its prevalence (Jaacks et al., 2019). The alteration of intestinal microbiome composition might play a critical role in the pathophysiology of obesity. Analysis of microbial composition in ob/ob mice (deficient for leptin), a mouse model of obesity, displayed an elevated abundance of *Firmicutes* and reduced level of *Bacteriodetes* (Ley et al., 2005; Turnbaugh et al., 2006). Consistent with this, obese patients also have increased *Firmicutes* and reduced *Bacteriodetes* abundances (Ley et al., 2005). Other extensive studies on gut microbial composition in obese mice showed a decrease in *Bifidobacteria* and an increase in *Halomonas* and *Sphingomonas* bacteria abundances (Waldram et al., 2009). Further, the proportions of *Lactobacillus paracasei* and *Akkermansia muciniphila* were reduced whereas *Lactobacillus reuteri* and *Lactobacillus gasseri* were enriched in the stool samples of obese individuals in comparison to healthy lean subjects (Million et al., 2012). Altogether, these studies suggest a potential implication of gut microbiota dysbiosis in the pathophysiology of obesity.

2.5. Cancer

During dysbiosis, specific pathogens proliferate rapidly and can trigger cancer development by adversely affecting the functioning of the host's gut and immune system or the host's metabolism (Rea et al., 2018). The implication of intestinal dysbiosis has been demonstrated for both local and distant tumor development (Sheflin et al., 2014). Disruption of microbial homeostasis is linked with several types of tumors, and it is estimated that nearly 20% of malignancies are triggered by microbial pathogens (Bhatt et al., 2017). The critical role of microbiome in the onset and progression of tumors has been described in several preclinical studies with germ-free mouse models (Nougayrède et al., 2006; Arthur et al., 2012). Additionally, the intestinal bacterial populations can overgrow during pathogenic infection-led dysbiosis and release huge quantities of toxins that can induce DNA breaks. Consequently, this results in genomic instability in cells and tumor onset and progression (Frisan, 2016; Zhang et al., 2021). Further, pathologic bacteria can interfere with pathways involved in DNA damage response and repair. For example, *Shigella flexineri* produces inositol phosphate phosphatase D (*ipGD*) and cysteine protease-like virulence gene A (*virA*) that promotes p53 degradation and enhances the chances of inserting mutations (Bergounioux et al., 2012). These studies highlight the involvement of microbiome in the pathogenesis of several malignancies.

2.6. Cardiovascular diseases (CVDs)

Cardiovascular diseases are the leading cause of mortality with a constant increase in prevalence worldwide (Martín-Sánchez et al., 2020). Although, the typical risk factors for CVDs are atherosclerosis, dyslipidemia, obesity, diabetes, and hypertension. Emerging research is highlighting the critical role of microbiota in cardiovascular health (Tang et al., 2013; Koeth et al., 2013). Interestingly, the modulation of *Firmicutes* to *Bacteroidetes* ratio is considered a potential risk factor for CVD development (Shin et al., 2015). Studies have shown the association of dysbiosis with the development of various CVDs (Tang et al., 2019; Ahmad et al., 2019). During dysbiosis, the integrity of the intestinal barrier is compromised resulting in increased levels of microbial components and metabolites in the circulation that can potentially trigger CVD development (Battson et al., 2018). Moreover, disruption of microbial composition also promotes the generation of pro-atherogenic metabolites in the intestine such as trimethylamine-N-oxide (TMAO) (Bu and Wang, 2018). Several infectious microorganisms including *Chlamydophila pneumoniae*, *Porphyromonas gingivalis*, *Helicobacter pylori*, Influenza A virus, Hepatitis C virus, *Cytomegalovirus*, and human immunodeficiency are also demonstrated to be linked with increased risk of CVD (Rosenfeld and Campbell, 2011). Infection associated plaque formation in atherosclerosis is driven by either directly infecting vessel wall or indirectly with a distant site infection that generates a systemic pro-inflammatory immune response (Jonsson and Bäckhed, 2017). Thus, dysbiosis significantly impacts the development of CVDs but their association is poorly understood and needs further investigations.

2.7. Central nervous system (CNS) disorders

Individuals with neurodegenerative disorders such as Parkinson's disease (PD) and Alzheimer's disease (AD) frequently display some gastrointestinal symptoms (Pfeiffer, 2003; Doraiswamy et al., 2002). Gut microbiotas influence the functioning of the brain by their impact on host's innate immune system (Sherwin et al., 2016). Furthermore, the newborns delivered through Cesarean section (C-section) showed less complex brain electrical activity in comparison to vaginally delivered neonates (Kim et al., 2003). Importantly, microbial diversity is also decreased in newborns delivered through C-section (Jakobsson et al., 2014) indicating a critical role for microbiome in the development of proper brain activity. Studies have shown modulation of microbial composition particularly increased abundances of *Prevotella* and *Akkermansia* whereas a decrease in *Lactobacillus species* proportion in PD patients relative to healthy individuals (Li et al., 2019). In addition, AD patients are reported to display an increase in *Bacteroidetes* and *Proteobacteria* and a decrease in *Firmicutes* and *Actinobacteria* in comparison to healthy controls (Vogt et al., 2017). Although these studies suggest a relationship between gut microbiome perturbation and neuronal disorders, further investigations are warranted to identify specific microbiota

that can be targeted to treat these disorders.

3. Limitations of conventional medicine in modulating gut microbiome

With the advent of the -omics technology the knowledge being gathered about the gut microbiome is continuously expanding. Although conventional medicine has long been prescribed for controlling and treating a wide spectrum of infectious and non-infectious diseases, recent research has shed light on the heavy price paid by the gut microbiome in return (Maier and Typas, 2017).

3.1. Conventional drug result in gut microbiome dysbiosis

Dysbiosis of the gut microbiome occurs when the composition and structure of the gut microbiome are altered and is associated with a variety of disorders and diseases. There is a growing body of evidence establishing that the administration of different classes of drugs including opioids, antibiotics, antidepressants, proton-pump inhibitors, anti-diabetics, chemotherapeutics, statins, steroids, and several other medications influence the gastrointestinal flora (Maier and Typas, 2017). Opioids are potent analgesics and are frequently prescribed for pain management. With its increased usage, opioid consumption has become a major health concern, causing problems of tolerance, dependence, and addiction (Rueda-Ruzafa et al., 2020). Several studies have shown that chronic exposure to opioids leads to opioid-induced gut microbiome dysbiosis (Rueda-Ruzafa et al., 2020; Ghosh et al., 2023; Meng et al., 2023; O'Sullivan et al., 2019; Thomas et al., 2022). Mouse studies have demonstrated that exposure to morphine increases the abundance of pathogenic bacteria such as *Flavobacterium*, *Enterococcus,* and *Clostridium* and a decrease in the beneficial *Bifidobacterium* and *Lactobacillus* sp. (Rueda-Ruzafa et al., 2020; Lee et al., 2018; Wang et al., 2018). Prolonged opioid use also leads to a decrease in butyrate producing *Firmicutes* bacteria, involved in luminal defense, that most likely results in an inflamed gut. (O'Sullivan et al., 2019; Guo et al., 2017). These reports also indicate that the observed inflammatory and microflora changes are major contributing factors in opioid addiction (O'Sullivan et al., 2019). Opioid withdrawal studies, involving abrupt cessation of opioids, have been shown to result in immunosuppression (Feng et al., 2006). More recent research has suggested that these opioid withdrawal effects are also induced because of a skewed *Firmicutes* to *Bacteroidetes* ratio (O'Sullivan et al., 2019).

Once considered a breakthrough in the history of medicine, the 'wicked' side of antibiotics has also been brought into the open. There is now enough evidence to prove that the overuse of antibiotics is associated with the onset of several disorders that are linked to the alteration of the gut microbiome (Blaser, 2016; Ianiro et al., 2016). Most antibiotics have a broad-spectrum action, affecting both the harmful and healthy microbes. Along with the impairment of harmful bacteria, they are also responsible for the development of antibiotic-resistant strains. Different classes of antibiotics alter the gut microbiome differently. Ianiro et al. published a review in 2016 compiling the effects of different classes of antibiotics on the gut microbiota detailed in Table 1 (Ianiro et al., 2016).

Antibiotic-induced gut dysbiosis is a phenomenon commonly associated with the overuse of antibiotics that impacts the diversity and richness of the gut microbiota (He et al., 2023). *Clostridium difficile*-induced colitis occurs commonly post-antibiotic treatment causing intestinal infection and diarrhea of varying severity and has emerged as a major health concern. A healthy gut can resist infection by *C. difficile* however patients administered with antibiotics have an impaired microbiome that-

-succumbs to the infection (Abt et al., 2016). Proton pump inhibitors, prescribed for treating gastrointestinal disorders like peptic ulcers and gastro-esophageal reflux, also alter the gut microbiome and predispose individuals to *C. difficile* infection (Freedberg et al., 2015; Matthew et al., 2016).

Table 1
Overview of the effects of different antibiotics on gut microbiota, according to their classes and excretion.

Antibiotic class	Antibiotic excretion	Effects on gut microbiota	References
Lincosamides **Clindamycin**	Main biliary excretion	↓Gram-positive aerobes and anaerobes ↑Resistance genes ↓ Bacteroides diversity	(Slimings and Riley, 2013; Rashid et al., 2015)
Macrolides **Clarithromycin** **Erythromycin**	Biliary excretion	↓Total bacterial diversity ↓*Actinobacteria* (including *Bifidobacteria*) ↓*Firmicutes* (mainly *Lactobacilli*) ↑*Bacteroidetes* ↑*Proteobacteria* ↓*Firmicutes* ↓*Actinobacteria* ↑*Proteobacteria*	(Jernberg et al., 2007; Jakobsson et al., 2010; Arboleya et al., 2016)
β-Lactams **Penicillin V** **Amoxicillin** **Ampicillin/ sulbactam** **Cephalosporins**	Main urinary excretion Partial (33–67%) biliary excretion[24]	No relevant changes ↓*Firmicutes* ↓*Actinobacteria* ↑*Proteobacteria* No relevant changes ↓Total bacterial richness ↓*Firmicutes* ↑*Bacteroidetes* ↑*Proteobacteria* ↓*Firmicutes* ↓*Actinobacteria* ↑*Proteobacteria* ↓Total bacterial richness ↓*Firmicutes* ↑*Bacteroidetes* ↑*Proteobacteria*	(Jakobsson et al., 2010; Arboleya et al., 2016; Korpela et al., 2016; Isanaka et al., 2016)
Fluoroquinolones **Ciprofloxacin** **Levofloxacin**	Partial biliary excretion	↓Bacterial diversity ↓Gram-negative facultative anaerobes ↑Gram-positive aerobes ↓Gram-negative facultative anaerobes ↓Gram-positive anaerobes	(Arboleya et al., 2016; Patel and Kaplan, 1984; INAGAKI et al., 1992)
Glycopeptides **Vancomycin**[a]		↓Total bacterial diversity ↓*Firmicutes* ↑*Proteobacteria*	(Jakobsson et al., 2010)

[a] Oral administration, vancomycin is not adsorbed when administered orally.

The effect of long-term medication on the gut microbiome is well established, however, a novel study by Jackson et al. has identified associations between the gut microbiome and other commonly prescribed medicines (Jackson et al., 2018a). Another high-throughput study performed by Maeir et al. involved screening of over 1000 drugs, of which a notable 24% of drugs exhibited anti-microbial activity against the 40 gut bacterial strains analyzed. A few drugs, mainly including antineoplastic agents such as daunorubicin, 5-fluorouracil, streptozotocin, and floxuridine were found to impact at least 50% of the bacterial strains studied (Maier et al., 2018). Other cancer treatment strategies such as chemotherapy, radiotherapy, surgery, etc. are also instrumental in altering the microbiome. GI (gastrointestinal) surgery, which also involves

pre-operative cleansing with antibiotics, changes the microflora of the gut and leads to further complications (Bachmann et al., 2017; Guyton and Alverdy, 2017). Metagenomic analysis of patients suffering from colitis post-administration of immune checkpoint inhibitors has shown alterations in microbial profile. Diarrhea, a common side-effect of chemotherapy, is also associated with microbial fluctuation observed post-treatment (Stringer et al., 2013).

Several anti-psychotics and anti-depressants prescribed for various psychological disorders including Major Depressive Disorder, bipolar disorder, and other anxiety disorders are known to have an anti-microbial effect, thereby impacting the gut microbiome (Bahr et al., 2015; Flowers et al., 2017; McGovern et al., 2019).

Gut dysbiosis makes individuals prone to several pathological conditions such as IBD, Crohn's disease, ulcerative colitis, diabetes, obesity, Parkinson's Disease, depression, etc. Therefore, a thorough research of the impact of various drugs on the microbiome is warranted to ascertain their effects on an individual. It is also imperative to explore alternate treatment strategies for treating various human ailments that help maintain the gut microbiome integrity.

3.2. *Suggested mechanism of action of drugs on the microbiome*

The drugs may impact the microbiome through two different modes of action which have been proposed. In the first mechanism, the drug may mediate the translocation of the microflora from other organs to the gut. For example, proton pump inhibitors decrease the acidity of the stomach thereby facilitating the movement of oral microbes to the gut resulting in dysbiosis. Such translocations may further lead to other diseased conditions, for example, an influx of oral microbes into the gut is suggested to play a role in colorectal cancer pathogenesis (Wing Yin et al., 2020). In the second mechanism, the alteration that the drugs cause to the gut environment may directly influence bacterial growth. This is observed frequently as in the case of antibiotics, opioids, etc. (Rinse et al., 2020).

3.3. *Impact of the gut microbiome on the therapeutic efficacy of drugs*

The interaction between the drugs and the microbiome is bi-directional: the drugs have a strong impact on the gut microbiome, but the microbiome can also heavily influence the action of drug (Maier and Typas, 2017; Rinse et al., 2020; Forslund et al., 2015; Jackson et al., 2018b). Several reports have shown the gut microbiome altering the drug availability and therapeutic efficacy. The metabolism of drugs by the microbial population can lead to the production of secondary metabolites that can be both beneficial and toxic. For example, a study by Klünemann et al. has reported the depletion of several structurally diverse drugs by the representative strains of the gut microbiome through bioaccumulation (Klünemann et al., 2021). The interaction between the microbiome and drugs is highly complex and dynamic and there is still a lot left to be unraveled. The field of pharmaco-microbiomics is just emerging and gathering a deeper understanding of how the gut microbiota metabolizes the drug will further enhance its therapeutic efficacy.

4. Phages as microbiota modulators to treat disease

In general, bacteriophages go through two different life cycles: the lytic cycle and the lysogenic cycle. Phage's attach to bacteria by attaching to a receptor found on their surface and injecting their genetic material into the cell (Step 1-2, in Fig. 2). For the phage to replicate its genetic material and produce progeny phages, the host cell provides the molecular building blocks and required enzymes such as phage-encoded proteins such as endolysin and holin lyse within the host cell (Step 3a, in Fig. 2). Holins are small proteins that accumulate in the cytoplasmic membrane of the host and allow endolysin to degrade peptidoglycan, allowing the progeny phage to escape (Step 4a, in Fig. 2) (Young and Blasi, 1995). Subsequently, in the external environment, lytic phage can infect and destroy all neighboring bacteria. In phage therapy, the ability of lytic phage to produce large numbers of progeny is an advantage. However, lytic phage is restricted to a narrow range of hosts and are capable of infecting only a few bacteria species. This limitation may be overcome using a phage cocktail. In a recent study, Elinav and colleagues report that a cocktail of five phages successfully targets a *K. pneumoniae* strain associated with inflammation in IBD, suppressing inflammation and disease in IBD models (Federici et al., 2022).

During lysogenic life cycle, temperate phage is not immediately capable of lysing the host cell; rather, their genome is inserted into the host chromosome at specific sites (*Step 3b, in* Fig. 3). The phage DNA

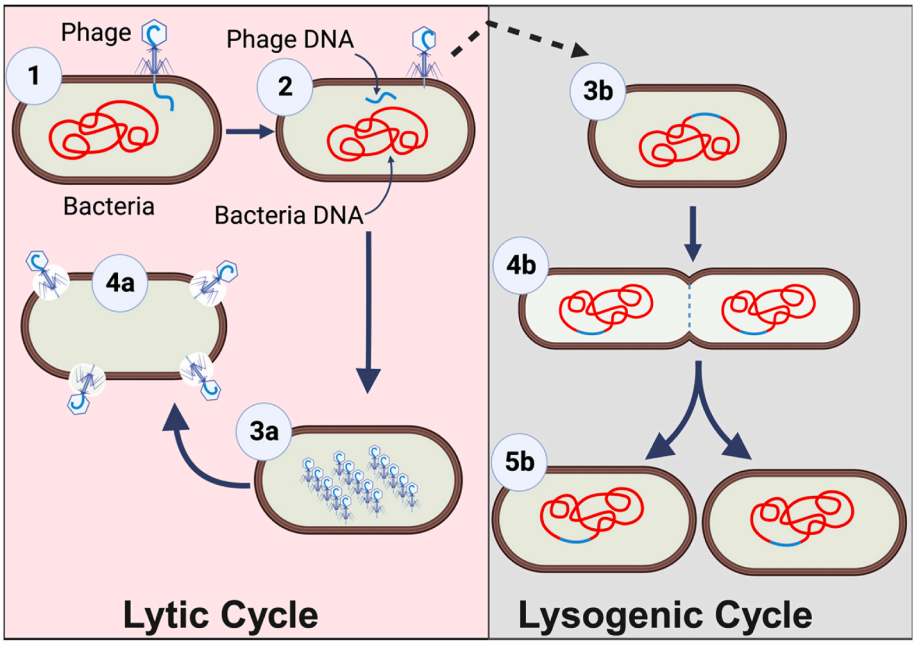

Fig. 2. The phage life cycle. Lytic phages undergo the lytic cycle, during which the host is lysed, and progeny phage are released into the environment. A temperate phage may undergo lytic or lysogenic cycles (Created with BioRender.com.).

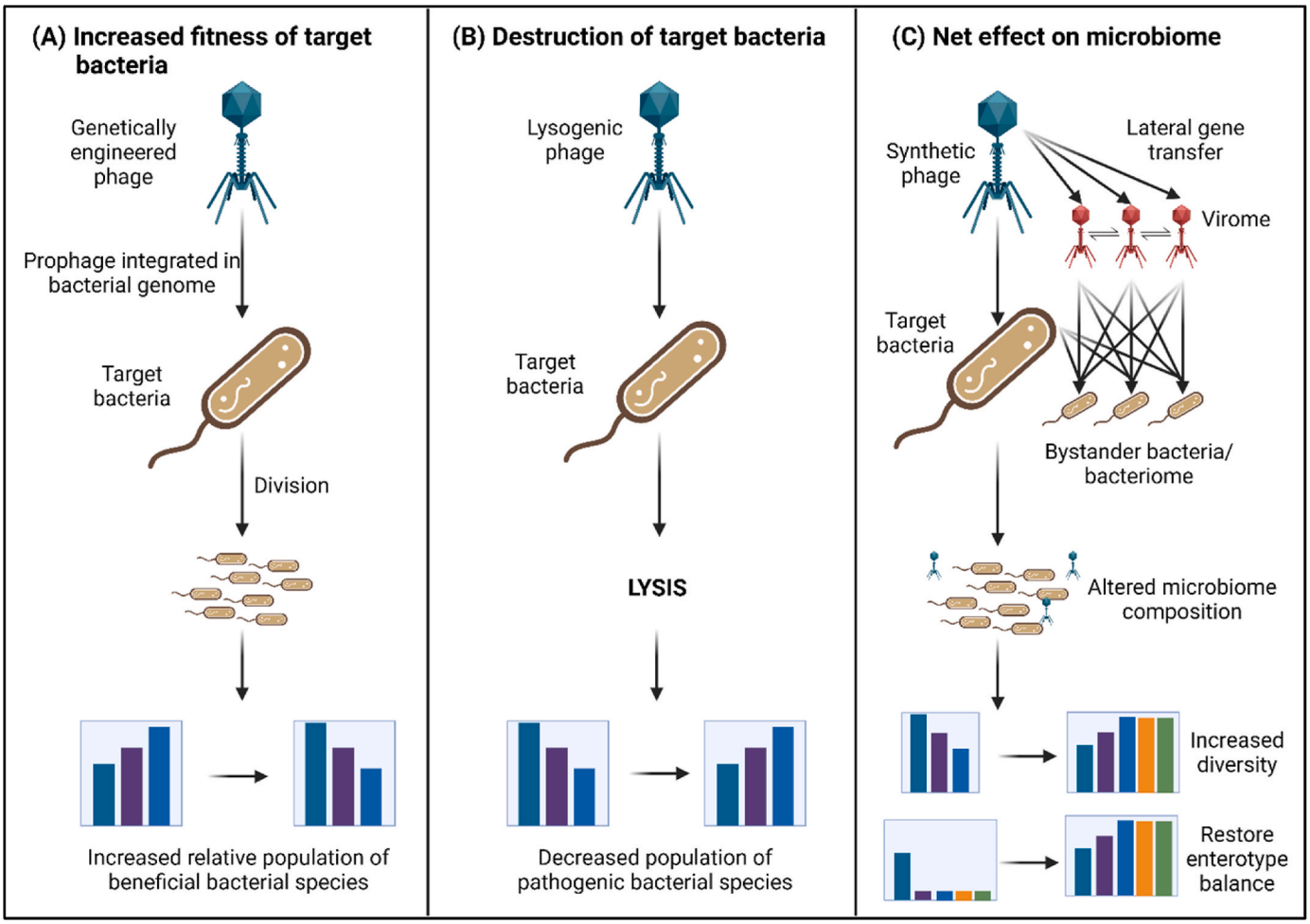

Fig. 3. Strategies for phage-induced modulation of the microbiome in disease states. A. Synthetic phage construct could induce incorporation of adaptive genetic elements into a specified bacterial species in the gut resulting in greater resilience to dysbiosis caused unintendedly by antibacterial treatment or during an infection. **B.** Programing and activation of the lysogenic switch in engineered phages can allow them to target and destroy specific bacterial populations i.e., a pathogenic bacterium while sparing the normal commensal species very accurately. **C.** Introduction of a synthetic phage can have multiple effects on both the virome and the bacteriome. The complex net effect can be predicted using cutting-edge computational modeling and might be a way to favorably alter metabolism to produce beneficial secondary metabolites; for instance, increasing production of antioxidants like polyphenols to combat neuroinflammatory disorders such as Alzheimer's disease (Kho and Lal, 2018). (Created with BioRender.com).

inserted into the host genome is referred to as a prophage, whereas the host cell that contains the prophage is known as a lysogen. Prophages are replicated together with the bacterial host genome, establishing a stable relationship (*Step 4b-5b, in* Fig. 2). It is important to note that one of the disadvantages associated with using temperate phages in phage therapy is that some of them insert their genome into the host chromosome and may lay dormant or alter the phenotype of the host. The lysogenic cycle can continue indefinitely unless the bacteria are exposed to stress or adverse environmental conditions. The induction signals vary among bacteriophage, but prophage is commonly induced when bacterial SOS responses are activated due to antibiotic treatment, oxidative stress, or DNA damage (Penades et al., 2015). Some phages rely on small molecules to communicate and execute lysis–lysogeny decisions (Erez et al., 2017).

We have previously discussed the connection between dysbiosis and various disease states, offering examples from different pathologies. In each case, dysbiosis either serves as a hallmark or directly contributes to pathogenesis. As previously illustrated, a lack of microbial diversity can create an environment in which pathogenic bacteria can thrive unchecked, leading to disease or interfering with the digestion and absorption of beneficial compounds that exacerbate certain disease states (Hsu et al., 2019). Dysregulation can result in an infectious state either because of the introduction of a foreign harmful species, or the abnormal proliferation of a commensal species that at regular ratios is a healthy part of the human flora (Meng et al., 2020). Beyond establishing dysregulations in microbiome homeostasis during disease, we can identify a general range of optimal microbiome compositions for healthy individuals (Fumagalli et al., 2023).

4.1. *Virome bacteriome synergy*

A healthy gut microbiome is generally characterized by a greater diversity of microbial species and a unique enterotype balance that varies between individuals. The virome, a component of the microbiome, exerts a significant influence on the bacteriome through both negative and positive modulation (Fig. 3) (Cao et al., 2022). Phage elements, for instance, have been shown to increase bacterial resistance and pathogenicity in numerous infections (Zuo et al., 2018). Consequently, antiviral therapy targeting these modulator phages could potentially serve as a viable therapeutic approach. Moreover, targeted phage therapy can promote a return to a normal microbiome enterotype and enhance diversity (Dixit et al., 2021). For instance, fecal virome transplantation (FVT) has been demonstrated to rectify dysbiosis resulting from antibiotic use, obesity, and bacterial infections (Draper

et al., 2020).

In one interesting study, fecal viral-like particles (VLPs) from lean mice were transplanted into obese mice maintained on a high-fat diet. The procedure resulted in a significant reduction in obesity as well as symptoms of Type 2 diabetes (Rasmussen et al., 2020). In a clinical study, FVT proved effective in alleviating symptoms in patients with recurrent *Clostridium difficile* infection (rCDI) (Kao et al., 2019). These observations post-FVT were concurrent with significant shifts in the gut microbiome including increased virome diversity and a decreased relative abundance of ileal mucosal Proteobacteria (Raeisi et al., 2023). Although these effects helped restore the subject's microbiome composition, similar studies note a persistent change in the recipient enterotype representing a shift to reflect the donor enterotype.

Introduction of phage constructs that induce specific adaptations in particular bacterial species has been observed. An example of this is the experiment by Veses-Garcia et al. (2015) in which the F24B phage increased the pH resistance of *E. coli* strain O157. It is feasible that synthetic phage could induce an adaptation conferring a selective advantage to a known commensal. Restoring the counts of a single bacterial species to pre-disease state levels may have a small balancing effect on dysbiosis. However, such a strategy could have a significant application in infections where increased counts of the causal microbe are associated with decreases in one or several specific commensal species. For instance, patients with autoimmune liver diseases like primary biliary cholangitis (PBC) and autoimmune hepatitis (AIH) show a marked increase in *Veillonella* sp., which is associated with a sharp decrease in regular *Fusobacterium counts* (Dixit et al., 2021). As the dynamics of such an interaction are nearly impossible to predict, no notable therapies have made use of this strategy.

4.2. Bacteriophage as a new antibiotic

Single-stranded RNA viruses of the family *Leviviridae*, single-stranded DNA viruses of family *Microviridae* and double-stranded DNA viruses of the order *Caudovirales* with the families of *Myoviridae*, *Siphoviridae*, and *Podoviridae* are mainly used bacteriophages for therapy (Dixit et al., 2021). While phage therapy can promote a healthy enterotype, it can also be engineered to target and eliminate specific pathogen populations. Yue et al. (Hu et al., 2018) showed that bacteriophage therapy was particularly successful in fighting *Salmonella* infection, proposing it could serve as a valuable antimicrobial therapeutic. Moreover, they showed that phage therapy had a synergistic effect when combined with the azithromycin antibiotic treatment. A similar synergistic effect is observed in combating *Pseudomonas* infection. Chaudhry et al. hypothesize that when the phage therapy is specifically introduced first it disrupts biofilm architecture thus allowing higher penetrance of the antibiotic (Chaudhry et al., 2017).

Technological advances in the last decade have allowed control over the induction of the lytic-lysogenic switch in the bacteriophage. In one study method, the CRISPR-Cas3 system was used to knock down lysogeny-related genes in *C. difficile* phages, successfully converting them into obligately virulent (lytic) types (Selle et al., 2020; Garneau et al., 2010). Subsequent experiments demonstrated these phages could effectively target and lyse *C. difficile* both *in vitro* and *in vivo* using a mouse model. In another study, researchers treated colorectal cancer in mice by loading phages targeting *Fusobacterium nucleatum*, a microbe associated with tumor development, and an anti-tumor drug into lipid nanoparticle constructs. The conjugated phages acted as guiding moieties delivering the nanoparticles precisely to the tumor, inhibiting the growth of *F. nucleatum*; while also promoting the proliferation of bacteria with anti-tumor effects like *Clostridium butyricum* (Zheng et al., 2019).

Phage levels can modulate the microbiome in disease states by interacting with the immune system, thereby affecting the immune response to dysbiosis or infection (Popescu et al., 2021). Abundant commensal phages at the dermis, bodily openings, and mucosal surfaces establish a line of defense against the introduction of foreign pathogens to the body, contributing to the innate immune system (Fernández-Tom et al., 2021). Beyond their direct role in defending against bacterial invasion at the mucin layers, phages interact with the human immune system to maintain immune homeostasis and influence the disease process. Several studies have revealed that phages can regulate the release of cytokines, enhancing opsonization and recognition of pathogens: guiding the action of T and B cells (Dery et al., 2021). Phage subunits have even been used as vaccines. In particular, the phage protein bacterial cell wall lysin identified in a phage that commonly targets methicillin-resistant *S. aureus* (MRSA) elicits a potent immune response and provides MRSA protection (Yang et al., 2018). The bacteriophage immune system influence has also been utilized in therapy for inflammatory and autoimmune diseases such as hidradenitis suppurative, a skin disease in which an inflammatory response is caused in part by dysbiosis of the skin microbiome (Bens et al., 2023).

4.3. Bacteriophage and microbiome in suppressing multi-drug resistance (MDR)

Phage therapy was historically dismissed due to the complexities of predicting pleiotropic effects, pharmacokinetics resulting from phage multiplication, and unknown immune interactions (Marongiu et al., 2022). With massive strides in technology, these same characteristics could make phage therapy a very attractive mode of treatment for multi-target multifactorial disease as well as personalized treatment (Lenneman et al., 2021a). In contrast to an antimicrobial drug which consists of a specific formulation, a phage displays genetic diversity that can be leveraged to quickly optimize therapies. Introducing a phage-based therapy presents not only a construct with pharmacologic effects but an enduring organism that continues to influence the metabolic landscape long after the initial dose (Abedon et al., 2021). A therapy that can continually adapt in acting to modulate the macrobiotic landscape once administered might have the potential to outpace perturbances caused by rapidly mutating MDR strains (Terwilliger et al., 2020). In addition, the engineering of phage strains over time can result in lines with greater lytic potential, therapeutic efficacy, and specificity to specific host environments.

In the era of rapidly evolving MDR bacterial strains, the need to find new ways to manipulate human bacterial ecosystems is quickly increasing. Phages and antibiotics have no similarities in the mechanisms they use to enact antibiotic effects, thus cross-resistance to combined therapies is unlikely to develop. Antibiotic-phage therapy has already been tested *in vivo* and has shown promise in preventing the evolution of phage-resistant clones (Van Nieuwenhuyse et al., 2022). The use of combined therapies has subsequentially shown activity against MDR biofilms that were unresponsive to antibiotics alone (Gan et al., 2022). Furthermore, new genetic and chemical engineering techniques are being applied to modify bacteriophages (Khambhati et al., 2023). Among the modifications are the ability to express toxin proteins and specific host recognition receptors. These actions allow for more precise modulation of bacteriophage effect on the microbiome, as well as rational design of combined therapies with other antimicrobials (Fig. 3) (Guo et al., 2021).

4.4. Hijacking the metabolome

High-coverage metabolomics technologies are allowing for a better understanding of the net metabolic impact of phage infection on bacterial metabolism. A growing body of research is leading to developing methodologies for discerning phage-specific effects in predicting end metabolite production. In one study on *P. aeruginosa* a phage-specific response was observed involving increased pyrimidine and sugar metabolism, leading to depletion (De Smet et al., 2016). Synthetic phages have shown the capacity to accurately target specific bacterial species within the gut microbiota (Guo et al., 2021; Lenneman et al.,

2021b), and with the ability to analyze metabolomics data the net effect on the microbiome and metabolite production can be discerned. A very clear example is demonstrated in one study where phage infection caused knockdown of genes in certain bacteria, leading to decreased production of secondary gut metabolites including tryptamine in one case and tyramine in another (Hsu et al., 2018, 2019).

Gut microbiota release metabolites and factors including hydrogen sulfide (H_2S) which inhibits complex IV of the electron transfer chain; reactive oxygen species (ROS) that can trigger inflammatory responses and increase cell oxidative stress; nitric oxide (NO) that can inhibit the tricarboxylic acid cycle (TCA); and SCFAs precursors for signaling molecules and energy production (Borbolis et al., 2023). All of these secondary bioactive compounds can directly affect mitochondria activity, and adenosine triphosphate (ATP) production, and alter the nuclear genome. Bacteriophages modulate microbiota quality and quantity and as discussed may be used to precisely increase or decrease counts of specific microbial species-altering the microbiota's secretome. Moreover, synthetic prophages carrying desired metabolite biosynthesis genes can be engineered to selectively target and genetically modify commensal bacteria species producing beneficial effects (Paule et al., 2018). By such methods, high levels of bioactive metabolites that decrease inflammation in disease states can be endogenously synthesized. A future step might be inducing the production of altered metabolites with expanded activity or more precise pharmacodynamics.

4.5. Clinical translation

A rise in phage therapy clinical reports has been seen since 2018 and experts assert these studies could support therapeutic safety (Petrovic et al., 2023). There has been a corresponding increase in funding for phage research and the initiation of several controlled clinical trials in the field (Abedon et al., 2021). Many countries have started to follow PHAGEFORCE study protocol which implements standards for therapy and experimentation including collection of patient and scientific data as well as expansion of phage banks (Onsea et al., 2021). Meanwhile, initiatives like the Tailored Antibacterials and Innovative Laboratories for Phage (Φ) Research (TAILΦR) at Baylor College of Medicine are attempting to come up with a standardized system to discover and evaluate phage "cocktails" that can be quickly validated for clinical use in personalized treatments (Terwilliger et al., 2020).

The use of phages as therapy has widely been discussed in Europe by the European Medicines Agency for ethical policy reasons since this therapy includes live medicine. In comparison, in Eastern European countries and Switzerland, phage therapy has been widely used for trials and therapies for a long time (Shim, 2023). The use of bacteriophages in Europe, Australia, and America is still experimental and raises questions for ethical and safety reasons.

5. Emerging technologies and the limitations of phage therapy

Bacteria though speculated to be at the lowermost strata of evolution are notoriously known to evolve constantly in response to their environment, striving for better survival. The current threat to the global healthcare system is the emergence of multidrug-resistant bacterial pathogenic strains. The overuse of antibiotics has led to an accelerated spread of antibiotic resistance across a multitude of bacterial species. The use of bacteriophages for the treatment of multidrug-resistant bacterial infections has gained attention over the recent decade as a possible alternative treatment method. However, certain factors remain as a limitation in the progress of Phage therapy.

5.1. Clinical trials of phage therapy

The outcome of clinical trials with different phage–bacteria combinations exhibit broad variation in efficacy among trial participants ranging from complete to nil clearance of bacteria, therefore it is difficult to predict the outcome of a treatment in individual cases. However, smaller trials and case-by-case treatments are currently in practice. Phage therapy of single patients is occasionally effective, but due to the severity of their infections, simultaneous antibiotic therapy is common (Nilsson, 2019).

Policies and regulations on the clinical application of phage therapy require a comprehensive regulatory framework as compared to antibiotics. This lack of standardized guidelines may lead to inconsistency in quality control, and safety assessment, potentially resulting in compromised trial efficacy. The vast repertoire of pharma companies using conventional antibiotics led to the present-day socioeconomic structures and drug regulation policies. These policies prove to be inadequate for the establishment of successful large-scale customized phage therapy. Further, using live phage cocktails as therapeutic agents raises ethical concerns encompassing plausible unintended consequences of phage interactions with non-target bacteria or the emergence of phage-resistant bacteria. Additionally, the safety of phage therapy in immunocompromised individuals, and aged population as a representation of vulnerable population require further investigations (Nilsson, 2019; Lin et al., 2022).

5.2. Pharmacokinetics and pharmacodynamics of phage therapy

Experimentalists designing phage therapy research mostly consider mathematical models and *in-vitro* experiments, which do not reflect all aspects of phage-bacteria interactions. These models work around parameters like free phages and uninfected bacterial titers, and the adsorption rate; the spatial distribution of phages and bacteria is to be ideally uniform, and the diffusion rate to be infinite. Efforts have been made to modify mathematical models, and encompass various in-vitro infection parameters like adsorption rate, latency times, and burst sizes of many phage-bacteria combinations for better simulation pharmacokinetics of phage therapy (Nilsson, 2019).

Phages are highly specific to particular bacterial strains, making it challenging to find a suitable phage for every bacterial infection. Meanwhile also limits its applicability to infections caused by single bacterial pathogens, whereas in a considerable number of cases, an infection involves a consortium of pathogenic bacterial strains (Lin et al., 2022).

Phages being significantly larger than antibiotics, this limits the administration dose as well as lowers the uptake and transportation rates, the protein nature of phages causes them to be phagocytized subsequently affecting the efficacy of a treatment. Another reason for the inefficient outcome of phage therapy might be a tendency for phages to bind to bacterial debris resulting from already lysed bacteria that may play a significant role in the inactivation of phages (Nilsson, 2019). A bacterial population is not a homogeneous cluster of equally susceptible cells. There is spatial heterogeneity due to bacteria biofilms or bacteria hiding in crevices of the blood vessel forming micro-colonies and ideal escape niches. There could be multiple factors influencing the efficacy of phage therapy, numerous anti-bacteriophage strategies are adopted by the bacteria, including adsorption inhibition, restriction enzyme modification systems, (CRISPR–Cas) system, abortion infection, and superinfection immunity (Nilsson, 2019; Lin et al., 2022; Shuwen and Kefeng, 2022; Jurado et al., 2022).

Adsorption resistance leads to a reduction in the interactions between bacteriophages and bacteria. Bacterial receptors vary naturally causing variation in the rate of adsorption within the population eventually leading to the evolutionary selection of bacteria exhibiting low adsorption rate. Bacteria also interfere with the biosynthesis of Phage particles via restriction-modification systems comprising endonucleases and methyltransferases. Bacteria with the help of endonucleases targets the short viral nucleotide sequence and simultaneously protects its own genome sequence from degrading with the help of methyltransferases. The Clustered regularly interspaced short palindromic repeats (CRISPR)-Cas systems are another machinery employed by the bacteria

to prevent phage infection. Virus-infected bacterial cells incorporate fragments of viral DNA in the spacer regions of their genome. These viral DNA fragments further work as an acquired memory response of the bacterium against new bouts of infections by the phage. Upon cell division, the immunological memory is passed down to daughter cells to process the CRISPR-RNA precursor transcripts to crCRIPSR-RNA transcripts that bind to Cas enzymes. The effector complexes hence formed explicitly identify complementary sequences (in the corresponding phage genome) and destroy the phage genome by the action of Cas enzymes (Nilsson, 2019; Lin et al., 2022; Shuwen and Kefeng, 2022; Jurado et al., 2022).

Bacteria often display phenotypic variance in their capacity to replicate the phage within. Depending on nutrient availability, bacteria may remain dormant as spores with low or negligible metabolism. At this point a phage infecting the bacteria shares the following fates of either hibernating or in a state pseudo lysogenic with stalled development, however upon nutrient replete conditions the phage might undergo a full lytic cycle extending the length of the infection cycle. Another phenomenon observed is superinfection, or infection of a prephage-infected cell by a new phage system, which often results in the inability of the second phage to inject and replicate its DNA, hence again an extension in the length of the infection period. The abortive infection is another mechanism adopted by bacteria by which the host bacteria induce its own death right after phage infection and strategically prior to the completion of phage reproduction and proliferation, thereby protecting neighboring uninfected host bacterial cells. The infected bacterial cells undergo leaky ATP flux and altered membrane potential to cause cell death thereby preventing phage dissemination to the neighboring uninfected susceptible bacterial cells. Conclusively, bacteria seem to have genetically controlled resistance mechanisms as well and they piggybank on phenotypic 'contingency plans' to quickly respond to phage infections (Nilsson, 2019; Lin et al., 2022; Shuwen and Kefeng, 2022; Jurado et al., 2022).

Additional aspects to take into consideration are the collateral effects of phage on human physiology, disease progression, and immune response. Bacteriophages often release bacterial toxins and intracellular components upon lysing host bacteria, which aggravates the progression of bacterial infections in certain instances leading to sepsis, and endotoxemia. Phage proteins might also be involved in immune-inflammatory disorders like colitis, IBD, rheumatoid arthritis, and allergic responses in humans (Nilsson, 2019; Lin et al., 2022; Shuwen and Kefeng, 2022; Jurado et al., 2022). Hence, therapeutics based on phages may be an exciting alternative to conventional antibacterial treatments in current clinical scenarios it however does come with arrays of limitations.

6. Future prospects

Phage therapy and gut microbiome manipulation are promising fields of research for a wide range of medical applications. Recent developments in phage therapy have shown great promise, including the combination of a phage and CRISPR-Cas system, as well as phage engineering. Using genetic engineering, it will be possible to modify phages to enhance their lysis ability, replication frequency, and tolerance to stress. A recent study has demonstrated that engineered bacteriophage efficiently kills infectious *Mycobacterium abscessus* in cystic fibrosis infection in the lungs (Dedrick et al., 2019). Further, personalized medicine is another promising prospect. Using metagenomic analysis, it is now possible to identify specific bacteria species present in everyone's gut microbiome. It paves the way for the development of personalized phage therapy treatments tailored to the microbiome of everyone. Since the gut microbiome plays a significant role in both health and disease, and antibiotic resistance is on the rise, phage therapy is a promising avenue for future investigation and clinical applications.

7. Conclusions

In recent years, bacteriophage therapy has gained a renewed level of attention due to its potential to selectively manipulate the gut microbiome. Phage therapy appears to be safe and effective, especially when compared to broad-spectrum antibiotics, but it remains a challenge to incorporate this therapy into mainstream treatment programs. There are several limitations to this approach, including the requirement to minimize indirect nonspecific immune reactions mediated by phages while focusing specifically on disease-associated commensals. Particularly when the targeted commensals are uncommon, this may be a daunting task. Furthermore, phage efficacy is another considerable factor that may require genetic engineering to ensure their safety. Phages may also be useful as adjuvants to other treatments or as adjuncts to antibiotics. It may be more beneficial to use such in combination with antibiotics to combat the rising incidence of multi-drug-resistant infections. In addition, exploiting different routes of phage administration may profoundly affect phage effector function and the resultant activation status of the host immune system. It would only be possible to develop immunotherapies in the future by leveraging the complex relationship among bacteria, phages, and the host.

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Abbreviations

(IBS)	Irritable bowel syndrome
(IBD)	Inflammatory bowel disease
(CRC)	Colorectal cancer
(CDI)	*Clostridium difficile* infection
(C.difficile)	Clostridium difficile
(FMT)	Fecal microbiota transplant
(USD)	Urinary stone disease
(FVT)	Fecal virome transplantation

References

Abedon, S.T., 2011. Lysis from without. Bacteriophage 1, 46–49.

Abedon, S.T., Danis-Wlodarczyk, K.M., Alves, D.R., 2021. Phage therapy in the 21st century: is there modern, clinical evidence of phage-mediated efficacy? Pharmaceuticals 14.
Abt, M.C., McKenney, P.T., Pamer, E.G., 2016. Clostridium difficile colitis: pathogenesis and host defence. Nat. Rev. Microbiol. 14, 609–620.
Afzaal, M., et al., 2022. Human gut microbiota in health and disease: unveiling the relationship. Front. Microbiol. 13, 999001.
Ahmad, A.F., Ward, N.C., Dwivedi, G., 2019. The gut microbiome and heart failure. Curr. Opin. Cardiol. 34, 225–232.
Allen, H.K., et al., 2011. Antibiotics in feed induce prophages in swine fecal microbiomes. mBio 2.
Arboleya, S., et al., 2016. Impact of prematurity and perinatal antibiotics on the developing intestinal microbiota: a functional inference study. Int. J. Mol. Sci. 17, 649.
Arthur, J.C., et al., 2012. Intestinal inflammation targets cancer-inducing activity of the microbiota. Science 338, 120–123.
Arumugam, M., et al., 2011. Enterotypes of the human gut microbiome. Nature 473, 174–180.
Bachmann, R., Leonard, D., Delzenne, N., Kartheuser, A., Cani, P.D., 2017. Novel insight into the role of microbiota in colorectal surgery. Gut 66, 738–749.
Bahr, S.M., et al., 2015. Use of the second-generation antipsychotic, risperidone, and secondary weight gain are associated with an altered gut microbiota in children. Transl. Psychiatry 5 e652-e652.
Battson, M.L., Lee, D.M., Weir, T.L., Gentile, C.L., 2018. The gut microbiota as a novel regulator of cardiovascular function and disease. J. Nutr. Biochem. 56, 1–15.
Bens, L., et al., 2023. Phage therapy for hidradenitis suppurativa: a unique challenge and possible opportunity for personalized treatment of a complex, inflammatory disease. Clin. Exp. 48, 1221–1229.
Bergounioux, J., et al., 2012. Calpain activation by the Shigella flexneri effector VirA regulates key steps in the formation and life of the bacterium's epithelial niche. Cell Host Microbe 11, 240–252.
Bhatt, A.P., Redinbo, M.R., Bultman, S.J., 2017. The role of the microbiome in cancer development and therapy. CA A Cancer J. Clin. 67, 326–344.
Blaser, M.J., 2016. Antibiotic use and its consequences for the normal microbiome. Science 352, 544–545.
Blaser, M.J., Falkow, S., 2009. What are the consequences of the disappearing human microbiota? Nat. Rev. Microbiol. 7, 887–894.
Borbolis, F., Mytilinaiou, E., Palikaras, K., 2023. The crosstalk between microbiome and mitochondrial homeostasis in neurodegeneration. Cells 12.
Breitbart, M., et al., 2003. Metagenomic analyses of an uncultured viral community from human feces. J. Bacteriol. 185, 6220–6223.
Britton, R.A., Young, V.B., 2014. Role of the intestinal microbiota in resistance to colonization by Clostridium difficile. Gastroenterology 146, 1547–1553.
Bu, J., Wang, Z., 2018. Cross-talk between gut microbiota and heart via the routes of metabolite and immunity. Gastroenterology research and practice 2018.
Cao, Z., et al., 2022. The gut virome: a new microbiome component in health and disease. EBioMedicine 81, 104113.
Casen, C., et al., 2015. Deviations in human gut microbiota: a novel diagnostic test for determining dysbiosis in patients with IBS or IBD. Aliment Pharmacol. Therapeut. 42, 71–83.
Chaudhry, W.N., et al., 2017. Synergy and order effects of antibiotics and phages in killing Pseudomonas aeruginosa biofilms. PLoS One 12, e0168615.
Chehri, M., et al., 2018. Case series of successful treatment with fecal microbiota transplant (FMT) oral capsules mixed from multiple donors even in patients previously treated with FMT enemas for recurrent Clostridium difficile infection. Medicine (Baltim.) 97, e11706.
Chen, B., Kim, J.J.-W., Zhang, Y., Du, L., Dai, N., 2018. Prevalence and predictors of small intestinal bacterial overgrowth in irritable bowel syndrome: a systematic review and meta-analysis. J. Gastroenterol. 53, 807–818.
Crusell, M.K.W., et al., 2018. Gestational diabetes is associated with change in the gut microbiota composition in third trimester of pregnancy and postpartum. Microbiome 6, 89.
Dedrick, R.M., et al., 2019. Engineered bacteriophages for treatment of a patient with a disseminated drug-resistant Mycobacterium abscessus. Nat. Med. 25, 730–733.
Dery, K.J., Górski, A., Międzybrodzki, R., Farmer, D.G., Kupiec-Weglinski, J.W., 2021. Therapeutic perspectives and mechanistic insights of phage therapy in allotransplantation. Transplantation 105, 1449–1458.
Dixit, K., Chaudhari, D., Dhotre, D., Shouche, Y., Saroj, S., 2021. Restoration of dysbiotic human gut microbiome for homeostasis. Life Sci. 278, 119622.
Doraiswamy, P.M., Leon, J., Cummings, J.L., Marin, D., Neumann, P.J., 2002. Prevalence and impact of medical comorbidity in Alzheimer's disease. J Gerontol A Biol Sci Med Sci 57, M173–M177.
Draper, L.A., et al., 2020. Autochthonous faecal viral transfer (FVT) impacts the murine microbiome after antibiotic perturbation. BMC Biol. 18, 173.
Drossman, D.A., 2006. The functional gastrointestinal disorders and the Rome III process. Gastroenterology 130, 1377–1390.
El-Salhy, M., Mazzawi, T., 2018. Fecal microbiota transplantation for managing irritable bowel syndrome. Expet Rev. Gastroenterol. Hepatol. 12, 439–445.
Erez, Z., et al., 2017. Communication between viruses guides lysis-lysogeny decisions. Nature 541, 488–493.
Everard, A., et al., 2011. Responses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic obese and diet-induced leptin-resistant mice. Diabetes 60, 2775–2786.
Everard, A., et al., 2013. Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. Proc. Natl. Acad. Sci. U. S. A. 110, 9066–9071.
Federici, S., et al., 2022. Targeted suppression of human IBD-associated gut microbiota commensals by phage consortia for treatment of intestinal inflammation. Cell 185, 2879–2898 e2824.
Feng, P., et al., 2006. Morphine withdrawal lowers host defense to enteric bacteria: spontaneous sepsis and increased sensitivity to oral Salmonella enterica serovar Typhimurium infection. Infect. Immun. 74, 5221–5226.
Fernández-Tomé, S., Ortega Moreno, L., Chaparro, M., Gisbert, J.P., 2021. Gut microbiota and dietary factors as modulators of the mucus layer in inflammatory bowel disease. Int. J. Mol. Sci. 22.
Flowers, S.A., Evans, S.J., Ward, K.M., McInnis, M.G., Ellingrod, V.L., 2017. Interaction between atypical antipsychotics and the gut microbiome in a bipolar disease cohort. Pharmacotherapy 37, 261–267.
Ford, A.C., et al., 2014. Efficacy of prebiotics, probiotics, and synbiotics in irritable bowel syndrome and chronic idiopathic constipation: systematic review and meta-analysis. Official journal of the American College of Gastroenterology| ACG 109, 1547–1561.
Ford, A.C., et al., 2018. American College of Gastroenterology monograph on management of irritable bowel syndrome. Official journal of the American College of Gastroenterology| ACG 113, 1–18.
Forslund, K., et al., 2015. Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. Nature 528, 262–266.
Frank, D.N., et al., 2007. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc. Natl. Acad. Sci. U. S. A. 104, 13780–13785.
Freedberg, D.E., et al., 2015. Proton pump inhibitors alter specific taxa in the human gastrointestinal microbiome: a crossover trial. Gastroenterology 149, 883–885.e889.
Frisan, T., 2016. Bacterial genotoxins: the long journey to the nucleus of mammalian cells. Biochim. Biophys. Acta Biomembr. 1858, 567–575.
Fukui, H., 2016. Increased intestinal permeability and decreased barrier function: does it really influence the risk of inflammation? Inflammatory intestinal diseases 1, 135–145.
Fumagalli, M.R., Saro, S.M., Tajana, M., Zapperi, S., La Porta, C.A.M., 2023. Quantitative analysis of disease-related metabolic dysregulation of human microbiota. iScience 26, 105868.
Gan, L., et al., 2022. Bacteriophage effectively rescues pneumonia caused by prevalent multidrug-resistant Klebsiella pneumoniae in the early stage. Microbiol. Spectr. 10, e0235822.
Garneau, J.E., et al., 2010. The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. Nature 468, 67–71.
Gevers, D., et al., 2014. The treatment-naive microbiome in new-onset Crohn's disease. Cell Host Microbe 15, 382–392.
Ghosh, N., et al., 2023. Morphine use induces gastric microbial dysbiosis driving gastric inflammation through TLR2 signalling which is attenuated by proton pump inhibition. Br. J. Pharmacol. 180, 1582–1596.
Gill, S.R., et al., 2006. Metagenomic analysis of the human distal gut microbiome. Science 312, 1355–1359.
Goerke, C., Koller, J., Wolz, C., 2006. Ciprofloxacin and trimethoprim cause phage induction and virulence modulation in Staphylococcus aureus. Antimicrob. Agents Chemother. 50, 171–177.
De Goffau, M.C., et al., 2013. Fecal microbiota composition differs between children with β-cell autoimmunity and those without. Diabetes 62, 1238–1244.
Goldin, B.R., Gualtieri, L.J., Moore, R.P., 1996. The effect of Lactobacillus GG on the initiation and promotion of DMH-induced intestinal tumors in the rat. Nutr. Cancer 25, 197–204.
González-Castro, A.M., et al., 2017. Mucosal pathobiology and molecular signature of epithelial barrier dysfunction in the small intestine in irritable bowel syndrome. J. Gastroenterol. Hepatol. 32, 53–63.
Grimaldi, R., et al., 2018. A prebiotic intervention study in children with autism spectrum disorders (ASDs). Microbiome 6, 133.
Groen, A.K., Nieuwdorp, M., 2017. An evaluation of the therapeutic potential of fecal microbiota transplantation to treat infectious and metabolic diseases. EMBO Mol. Med. 9, 1–3.
Guo, S., et al., 2017. Secretions of Bifidobacterium infantis and Lactobacillus acidophilus protect intestinal epithelial barrier function. J. Pediatr. Gastroenterol. Nutr. 64, 404–412.
Guo, D., et al., 2021. Genetic and chemical engineering of phages for controlling multidrug-resistant bacteria. Antibiotics 10.
Guyton, K., Alverdy, J.C., 2017. The gut microbiota and gastrointestinal surgery. Nat. Rev. Gastroenterol. Hepatol. 14, 43–54.
Hadrich, D., 2018. Microbiome research is becoming the key to better understanding health and nutrition. Front. Genet. 9, 212.
Halvorson, H.A., Schlett, C.D., Riddle, M.S., 2006. Postinfectious irritable bowel syndrome—a meta-analysis. Official journal of the American College of Gastroenterology| ACG 101, 1894–1899.
Hand, T.W., Vujkovic-Cvijin, I., Ridaura, V.K., Belkaid, Y., 2016. Linking the microbiota, chronic disease, and the immune system. Trends Endocrinol. Metabol. 27, 831–843.
Hawrelak, J.A., Myers, S.P., 2004. The causes of intestinal dysbiosis: a review. Alternative Med. Rev. 9, 180–197.
He, M., Shi, J., Liu, A., Xu, Y.J., Liu, Y., 2023. Antibiotic-induced gut microbiota dysbiosis altered host metabolism. Mol Omics 19, 330–339.
Henrot, C., Petit, M.A., 2022. Signals triggering prophage induction in the gut microbiota. Mol. Microbiol. 118, 494–502.
Hsu, B.B., et al., 2018. Bacteriophages dynamically modulate the gut microbiota and metabolome. bioRxiv.
Hsu, B.B., et al., 2019. Dynamic modulation of the gut microbiota and metabolome by bacteriophages in a mouse model. Cell Host Microbe 25, 803–814.e805.

Hu, Y.O.O., et al., 2018. Bacteriophages synergize with the gut microbial community to combat *Salmonella*. mSystems 3. https://doi.org/10.1128/msystems.00119-00118.
Hu, J., Ye, H., Wang, S., Wang, J., Han, D., 2021. Prophage activation in the intestine: insights into functions and possible applications. Front. Microbiol. 12, 785634.
Hughes, R.L., Holscher, H.D., 2021. Fueling gut microbes: a review of the interaction between diet, exercise, and the gut microbiota in athletes. Adv. Nutr. 12, 2190–2215.
Human, C., 2012. Microbiome Project, Structure, function and diversity of the healthy human microbiome. Nature 486, 207–214.
Ianiro, G., Tilg, H., Gasbarrini, A., 2016. Antibiotics as deep modulators of gut microbiota: between good and evil. Gut 65, 1906–1915.
Inagaki, Y., Nakaya, R., Chida, T., Hashimoto, S., 1992. The effect of levofloxacin, an optically-active isomer of ofloxacin, on fecal microflora in human volunteers. Jpn. J. Antibiot. 45, 241–252.
Ionescu, R.F., Enache, R.M., Cretoiu, S.M., Gaspar, B.S., 2022. Gut microbiome changes in gestational diabetes. Int. J. Mol. Sci. 23.
Isanaka, S., et al., 2016. Routine amoxicillin for uncomplicated severe acute malnutrition in children. N. Engl. J. Med. 374, 444–453.
Jaacks, L.M., et al., 2019. The obesity transition: stages of the global epidemic. Lancet Diabetes Endocrinol. 7, 231–240.
Jackson, M.A., et al., 2018a. Gut microbiota associations with common diseases and prescription medications in a population-based cohort. Nat. Commun. 9, 2655.
Jackson, M.A., et al., 2018b. Gut microbiota associations with common diseases and prescription medications in a population-based cohort. Nat. Commun. 9, 2655.
Jakobsson, H.E., et al., 2010. Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. PLoS One 5, e9836.
Jakobsson, H.E., et al., 2014. Decreased gut microbiota diversity, delayed Bacteroidetes colonisation and reduced Th1 responses in infants delivered by caesarean section. Gut 63, 559–566.
Jalanka, J., Salonen, A., Fuentes, S., de Vos, W.M., 2015. Microbial signatures in post-infectious irritable bowel syndrome–toward patient stratification for improved diagnostics and treatment. Gut Microb. 6, 364–369.
Jeffery, I.B., et al., 2012. An irritable bowel syndrome subtype defined by species-specific alterations in faecal microbiota. Gut 61, 997–1006.
Jeffery, I., et al., 2016. 260 alterations in the microbiota in irritable bowel syndrome; A comparison of two geographically distinct cohorts. Gastroenterology 150, S63.
Jernberg, C., Löfmark, S., Edlund, C., Jansson, J.K., 2007. Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. ISME J. 1, 56–66.
Jonsson, A.L., Bäckhed, F., 2017. Role of gut microbiota in atherosclerosis. Nat. Rev. Cardiol. 14, 79–87.
Jurado, A., Fernández, L., Rodríguez, A., García, P., 2022. Understanding the mechanisms that drive phage resistance in staphylococci to prevent phage therapy failure. Viruses 14.
Kao, D.H., Roach, B., Walter, J., Lobenberg, R., Wong, K., 2019. A51 effect of lyophilized sterile fecal filtrate vs lyophilized donor stool on recurrent CLOSTRIDIUM difficile infection (RCDI): preliminary results from a randomized, double-blind pilot study. Journal of the Canadian Association of Gastroenterology 2, 101–102.
Kaplan, G.G., 2015. The global burden of IBD: from 2015 to 2025. Nat. Rev. Gastroenterol. Hepatol. 12, 720–727.
Karin, M., Lawrence, T., Nizet, V., 2006. Innate immunity gone awry: linking microbial infections to chronic inflammation and cancer. Cell 124, 823–835.
Khambhati, K., et al., 2023. Phage engineering and phage-assisted CRISPR-Cas delivery to combat multidrug-resistant pathogens. Bioeng Transl Med 8, e10381.
Kho, Z.Y., Lal, S.K., 2018. The human gut microbiome - a potential controller of wellness and disease. Front. Microbiol. 9, 1835.
Kim, H.-R., et al., 2003. Delivery modes and neonatal EEG: spatial pattern analysis. Early Hum. Dev. 75, 35–53.
Klünemann, M., et al., 2021. Bioaccumulation of therapeutic drugs by human gut bacteria. Nature 597, 533–538.
Knowles, B., et al., 2016. Lytic to temperate switching of viral communities. Nature 531, 466–470.
Koeth, R.A., et al., 2013. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. Nat. Med. 19, 576–585.
Korpela, K., et al., 2016. Intestinal microbiome is related to lifetime antibiotic use in Finnish pre-school children. Nat. Commun. 7, 10410.
Kostic, A.D., Xavier, R.J., Gevers, D., 2014. The microbiome in inflammatory bowel disease: current status and the future ahead. Gastroenterology 146, 1489–1499.
Kostic, A.D., et al., 2015. The dynamics of the human infant gut microbiome in development and in progression toward type 1 diabetes. Cell Host Microbe 17, 260–273.
Lacy, B.E., et al., 2016. Bowel disorders. Gastroenterology 150, 1393–1407.
Lee, K., et al., 2018. The gut microbiota mediates reward and sensory responses associated with regimen-selective morphine dependence. Neuropsychopharmacology 43, 2606–2614.
Lee, C.B., Chae, S.U., Jo, S.J., Jerng, U.M., Bae, S.K., 2021. The relationship between the gut microbiome and metformin as a key for treating type 2 diabetes mellitus. Int. J. Mol. Sci. 22.
Lenneman, B.R., Fernbach, J., Loessner, M.J., Lu, T.K., Kilcher, S., 2021a. Enhancing phage therapy through synthetic biology and genome engineering. Curr. Opin. Biotechnol. 68, 151–159.
Lenneman, B.R., Fernbach, J., Loessner, M.J., Lu, T.K., Kilcher, S., 2021b. Enhancing phage therapy through synthetic biology and genome engineering. Curr. Opin. Biotechnol. 68, 151–159.
Leone, V., Chang, E.B., Devkota, S., 2013. Diet, microbes, and host genetics: the perfect storm in inflammatory bowel diseases. J. Gastroenterol. 48, 315–321.
Levy, M., Kolodziejczyk, A.A., Thaiss, C.A., Elinav, E., 2017. Dysbiosis and the immune system. Nat. Rev. Immunol. 17, 219–232.
Ley, R.E., et al., 2005. Obesity alters gut microbial ecology. Proc. Natl. Acad. Sci. USA 102, 11070–11075.
Li, J., Zhu, W., Liu, W., Wu, Y., Wu, B., 2016. Rifaximin for irritable bowel syndrome: a meta-analysis of randomized placebo-controlled trials. Medicine 95 (4), e2534.
Li, C., et al., 2019. Gut microbiota differs between Parkinson's disease patients and healthy controls in northeast China. Front. Mol. Neurosci. 12, 171.
Lin, D.M., Koskella, B., Lin, H.C., 2017. Phage therapy: an alternative to antibiotics in the age of multi-drug resistance. World J. Gastrointest. Pharmacol. Therapeut 8, 162–173.
Lin, J., Du, F., Long, M., Li, P., 2022. Limitations of phage therapy and corresponding optimization strategies: a review. Molecules 27.
Liu, X., et al., 2018. Intestinal epithelial cell-derived LKB1 suppresses colitogenic microbiota. J. Immunol. 200, 1889–1900.
Lozupone, C.A., Stombaugh, J.I., Gordon, J.I., Jansson, J.K., Knight, R., 2012. Diversity, stability and resilience of the human gut microbiota. Nature 489, 220–230.
Lynch, S.V., Pedersen, O., 2016. The human intestinal microbiome in health and disease. N. Engl. J. Med. 375, 2369–2379.
Machiels, K., et al., 2014. A decrease of the butyrate-producing species Roseburia hominis and Faecalibacterium prausnitzii defines dysbiosis in patients with ulcerative colitis. Gut 63, 1275–1283.
Maier, L., Typas, A., 2017. Systematically investigating the impact of medication on the gut microbiome. Curr. Opin. Microbiol. 39, 128–135.
Maier, L., et al., 2018. Extensive impact of non-antibiotic drugs on human gut bacteria. Nature 555, 623–628.
Makker, J., Chilimuri, S., Bella, J.N., 2015. Genetic epidemiology of irritable bowel syndrome. World J. Gastroenterol.: WJG 21, 11353–11361.
Mardinoglu, A., Boren, J., Smith, U., 2016. Confounding effects of metformin on the human gut microbiome in type 2 diabetes. Cell Metabol. 23, 10–12.
Mariño, E., et al., 2017. Gut microbial metabolites limit the frequency of autoimmune T cells and protect against type 1 diabetes. Nat. Immunol. 18, 552–562.
Marongiu, L., Burkard, M., Lauer, U.M., Hoelzle, L.E., Venturelli, S., 2022. Reassessment of historical clinical trials supports the effectiveness of phage therapy. Clin. Microbiol. Rev. 35, e0006222.
Martín-Sánchez, F.J., et al., 2020. Identification of Senior at Risk scale predicts 30-day mortality among older patients with acute heart failure. Med. Intensiva 44, 9–17.
Matthew, A.J., et al., 2016. Proton pump inhibitors alter the composition of the gut microbiota. Gut 65, 749.
McGovern, A.S., Hamlin, A.S., Winter, G., 2019. A review of the antimicrobial side of antidepressants and its putative implications on the gut microbiome. Aust. N. Z. J. Psychiatr. 53, 1151–1166.
Medzhitov, R., 2008. Origin and physiological roles of inflammation. Nature 454, 428–435.
Menees, S., Chey, W., 2018. The gut microbiome and irritable bowel syndrome. F1000Research 7.
Meng, X., et al., 2020. Gut dysbacteriosis and intestinal disease: mechanism and treatment. J. Appl. Microbiol. 129, 787–805.
Meng, J., et al., 2023. Opioid-induced microbial dysbiosis disrupts irinotecan (CPT-11) metabolism and increases gastrointestinal toxicity in a murine model. Br. J. Pharmacol. 180, 1362–1378.
Menke, A., Casagrande, S., Geiss, L., Cowie, C.C., 2015. Prevalence of and trends in diabetes among adults in the United States, 1988-2012. JAMA 314, 1021–1029.
Million, M., et al., 2012. Obesity-associated gut microbiota is enriched in Lactobacillus reuteri and depleted in Bifidobacterium animalis and Methanobrevibacter smithii. Int. J. Obes. 36, 817–825.
Minot, S., et al., 2011. The human gut virome: inter-individual variation and dynamic response to diet. Genome Res. 21, 1616–1625.
Moelling, K., Broecker, F., Willy, C., 2018. A wake-up call: we need phage therapy now. Viruses 10.
Moloney, R.D., et al., 2016. Stress and the microbiota–gut–brain axis in visceral pain: relevance to irritable bowel syndrome. CNS Neurosci. Ther. 22, 102–117.
Moreno-Gallego, J.L., et al., 2019. Virome diversity correlates with intestinal microbiome diversity in adult monozygotic twins. Cell Host Microbe 25, 261–272 e265.
Moser, G., Fournier, C., Peter, J., 2018. Intestinal microbiome-gut-brain axis and irritable bowel syndrome. Wien Med. Wochenschr. 168, 62–66 (1946).
Ng, M., et al., 2014. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 384, 766–781.
Ng, S.C., et al., 2017. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. Lancet 390, 2769–2778.
Ni, J., Wu, G.D., Albenberg, L., Tomov, V.T., 2017. Gut microbiota and IBD: causation or correlation? Nat. Rev. Gastroenterol. Hepatol. 14, 573–584.
Van Nieuwenhuyse, B., et al., 2022. Bacteriophage-antibiotic combination therapy against extensively drug-resistant Pseudomonas aeruginosa infection to allow liver transplantation in a toddler. Nat. Commun. 13, 5725.
Nilsson, A.S., 2019. Pharmacological limitations of phage therapy. Ups. J. Med. Sci. 124, 218–227.
Nougayrède, J.-P., et al., 2006. Escherichia coli induces DNA double-strand breaks in eukaryotic cells. Science 313, 848–851.
Öhman, L., Simrén, M., 2010. Pathogenesis of IBS: role of inflammation, immunity and neuroimmune interactions. Nat. Rev. Gastroenterol. Hepatol. 7, 163–173.

Oh, J.H., et al., 2019. Prophages in Lactobacillus reuteri are associated with fitness trade-offs but can increase competitiveness in the gut ecosystem. Appl. Environ. Microbiol. 86.
Onsea, J., et al., 2021. Bacteriophage therapy for difficult-to-treat infections: the implementation of a multidisciplinary phage task force (the PHAGEFORCE study protocol). Viruses 13.
O'Sullivan, S.J., et al., 2019. Single-cell glia and neuron gene expression in the central amygdala in opioid withdrawal suggests inflammation with correlated gut dysbiosis. Front. Neurosci. 13.
Patel, I., Kaplan, S., 1984. Pharmacokinetic profile of ceftriaxone in man. Am. J. Med. 77, 17–25.
Paule, A., Frezza, D., Edeas, M., 2018. Microbiota and phage therapy: future challenges in medicine. Med. Sci. 6, 86.
Pellissier, S., Bonaz, B., 2017. The place of stress and emotions in the irritable bowel syndrome. Vitam. Horm. 103, 327–354.
Penades, J.R., Chen, J., Quiles-Puchalt, N., Carpena, N., Novick, R.P., 2015. Bacteriophage-mediated spread of bacterial virulence genes. Curr. Opin. Microbiol. 23, 171–178.
Petrovic Fabijan, A., Iredell, J., Danis-Wlodarczyk, K., Kebriaei, R., Abedon, S.T., 2023. Translating phage therapy into the clinic: recent accomplishments but continuing challenges. PLoS Biol. 21, e3002119.
Pfeiffer, R.F., 2003. Gastrointestinal dysfunction in Parkinson's disease. Lancet Neurol. 2, 107–116.
Pittayanon, R., et al., 2019. Gut microbiota in patients with irritable bowel syndrome-A systematic review. Gastroenterology 157, 97–108.
Popescu, M., Van Belleghem, J.D., Khosravi, A., Bollyky, P.L., 2021. Bacteriophages and the immune system. Annu Rev Virol 8, 415–435.
Powell, N., Walker, M.M., Talley, N.J., 2017. The mucosal immune system: master regulator of bidirectional gut–brain communications. Nat. Rev. Gastroenterol. Hepatol. 14, 143–159.
Raeisi, H., et al., 2023. Emerging applications of phage therapy and fecal virome transplantation for treatment of Clostridioides difficile infection: challenges and perspectives. Gut Pathog. 15, 21.
Rangel, I., et al., 2015. The relationship between faecal-associated and mucosal-associated microbiota in irritable bowel syndrome patients and healthy subjects. Aliment. Pharmacol. Ther. 42, 1211–1221.
Rashid, M.-U., et al., 2015. Determining the long-term effect of antibiotic administration on the human normal intestinal microbiota using culture and pyrosequencing methods. Clin. Infect. Dis. 60, S77–S84.
Rasmussen, T.S., et al., 2020. Faecal virome transplantation decreases symptoms of type 2 diabetes and obesity in a murine model. Gut 69, 2122–2130.
Rea, D., et al., 2018. Microbiota effects on cancer: from risks to therapies. Oncotarget 9, 17915–17927.
Reyes, A., et al., 2010. Viruses in the faecal microbiota of monozygotic twins and their mothers. Nature 466, 334–338.
Rinse, K.W., Alexandra, Z., Jingyuan, F., 2020. Interaction between drugs and the gut microbiome. Gut 69, 1510.
Rosenfeld, M.E., Campbell, L.A., 2011. Pathogens and atherosclerosis: update on the potential contribution of multiple infectious organisms to the pathogenesis of atherosclerosis. Thromb. Haemostasis 106, 858–867.
Rueda-Ruzafa, L., et al., 2020. Opioid system influences gut-brain axis: dysbiosis and related alterations. Pharmacol. Res. 159, 104928.
Schnabel, R.B., et al., 2015. 50 year trends in atrial fibrillation prevalence, incidence, risk factors, and mortality in the Framingham Heart Study: a cohort study. Lancet 386, 154–162.
Selle, K., et al., 2020. In vivo targeting of clostridioides difficile using phage-delivered CRISPR-cas3 antimicrobials. mBio 11.
Shao, Y., Wang, I.N., 2008. Bacteriophage adsorption rate and optimal lysis time. Genetics 180, 471–482.
Sheflin, A.M., Whitney, A.K., Weir, T.L., 2014. Cancer-promoting effects of microbial dysbiosis. Curr. Oncol. Rep. 16, 1–9.
Sherwin, E., Rea, K., Dinan, T.G., Cryan, J.F., 2016. A gut (microbiome) feeling about the brain. Curr. Opin. Gastroenterol. 32, 96–102.
Shi, N., Li, N., Duan, X., Niu, H., 2017. Interaction between the gut microbiome and mucosal immune system. Military Medical Research 4, 14.
Shim, H., 2023. Three innovations of next-generation antibiotics: evolvability, specificity, and non-immunogenicity. Antibiotics 12.
Shin, N.-R., Whon, T.W., Bae, J.-W., 2015. Proteobacteria: microbial signature of dysbiosis in gut microbiota. Trends Biotechnol. 33, 496–503.
Shuwen, H., Kefeng, D., 2022. Intestinal phages interact with bacteria and are involved in human diseases. Gut Microb. 14, 2113717.
Slimings, C., Riley, T.V., 2013. Antibiotics and hospital-acquired Clostridium difficile infection: update of systematic review and meta-analysis. J. Antimicrob. Chemother. 69, 881–891.
De Smet, J., et al., 2016. High coverage metabolomics analysis reveals phage-specific alterations to Pseudomonas aeruginosa physiology during infection. ISME J. 10, 1823–1835.
So, D., et al., 2018. Dietary fiber intervention on gut microbiota composition in healthy adults: a systematic review and meta-analysis. Am. J. Clin. Nutr. 107, 965–983.
Sokol, H., et al., 2009. Low counts of Faecalibacterium prausnitzii in colitis microbiota. Inflamm. Bowel Dis. 15, 1183–1189.
St-Pierre, F., Endy, D., 2008. Determination of cell fate selection during phage lambda infection. Proc. Natl. Acad. Sci. U. S. A. 105, 20705–20710.
Stringer, A.M., et al., 2013. Biomarkers of chemotherapy-induced diarrhoea: a clinical study of intestinal microbiome alterations, inflammation and circulating matrix metalloproteinases. Support. Care Cancer 21, 1843–1852.
Sundin, J., Öhman, L., Simrén, M., 2017. Understanding the gut microbiota in inflammatory and functional gastrointestinal diseases. Psychosom. Med. 79, 857–867.
Suryavanshi, M.V., et al., 2016. Hyperoxaluria leads to dysbiosis and drives selective enrichment of oxalate metabolizing bacterial species in recurrent kidney stone endures. Sci. Rep. 6, 34712.
Suzuki, T., et al., 1985. In Immune-Deficient Animals in Biomedical Research. Karger Publishers, pp. 112–116.
Tang, W.H.W., et al., 2013. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. N. Engl. J. Med. 368, 1575–1584.
Tang, W.H.W., Bäckhed, F., Landmesser, U., Hazen, S.L., 2019. Intestinal microbiota in cardiovascular health and disease: JACC state-of-the-art review. J. Am. Coll. Cardiol. 73, 2089–2105.
Terwilliger, A.L., et al., 2020. Tailored antibacterials and innovative Laboratories for phage (Φ) research: personalized infectious disease medicine for the most vulnerable at-risk patients. Phage (New Rochelle) 1, 66–74.
Thomas, K.R., et al., 2022. Pain and opioid-induced gut microbial dysbiosis. Biomedicines 10, 1815.
Turnbaugh, P.J., et al., 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 444, 1027–1031.
Turnbaugh, P.J., et al., 2009. A core gut microbiome in obese and lean twins. Nature 457, 480–484.
Valentin, N., et al., 2017. Potential mechanisms of effects of serum-derived bovine immunoglobulin/protein isolate therapy in patients with diarrhea-predominant irritable bowel syndrome. Physiological reports 5, e13170.
Vatanen, T., et al., 2018. The human gut microbiome in early-onset type 1 diabetes from the TEDDY study. Nature 562, 589–594.
Veses-Garcia, M., et al., 2015. Transcriptomic analysis of Shiga-toxigenic bacteriophage carriage reveals a profound regulatory effect on acid resistance in Escherichia coli. Appl. Environ. Microbiol. 81, 8118–8125.
Vogt, N.M., et al., 2017. Gut microbiome alterations in Alzheimer's disease. Sci. Rep. 7, 13537.
Waldram, A., et al., 2009. Top-down systems biology modeling of host metabotype–microbiome associations in obese rodents. J. Proteome Res. 8, 2361–2375.
Wang, I.N., 2006. Lysis timing and bacteriophage fitness. Genetics 172, 17–26.
Wang, F., et al., 2018. Morphine induces changes in the gut microbiome and metabolome in a morphine dependence model. Sci. Rep. 8, 3596.
Wing Yin, C., Chun-Ying, W., Jun, Y., 2020. The role of gut microbiota in cancer treatment: friend or foe? Gut 69, 1867.
Wu, H., et al., 2017. Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. Nat. Med. 23, 850–858.
Yang, H., et al., 2018. Staphylococcus aureus virulence attenuation and immune clearance mediated by a phage lysin-derived protein. EMBO J. 37.
Young, R., Blasi, U., 1995. Holins: form and function in bacteriophage lysis. FEMS Microbiol. Rev. 17, 191–205.
Zhang, Y., et al., 2016. Effects of probiotic type, dose and treatment duration on irritable bowel syndrome diagnosed by Rome III criteria: a meta-analysis. BMC Gastroenterol. 16, 1–11.
Zhang, W., et al., 2021. Gut microbiota-derived metabolites in colorectal cancer: the bad and the challenges. Front. Oncol. 11, 739648.
Zheng, D.W., et al., 2019. Phage-guided modulation of the gut microbiota of mouse models of colorectal cancer augments their responses to chemotherapy. Nat. Biomed. Eng. 3, 717–728.
Zhong, W., et al., 2019. Distinct microbial populations exist in the mucosa-associated microbiota of diarrhea predominant irritable bowel syndrome and ulcerative colitis. J. Clin. Gastroenterol. 53, 660–672.
Zmora, N., et al., 2018. Personalized gut mucosal colonization resistance to empiric probiotics is associated with unique host and microbiome features. Cell 174, 1388–1405 e1321.
Zuo, T., et al., 2018. Bacteriophage transfer during faecal microbiota transplantation in Clostridium difficile infection is associated with treatment outcome. Gut 67, 634–643.